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## TOXIC BLUE-GREEN ALGAE IN SASKATCHEWAN

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## Introduction

IN INLAND WATERS, blue-green algae have frequently been held responsible for sickness and death of mammals, waterfowl, and fish. Gastrointestinal disorders in man have been suspected to be of blue-green algal origin (27). The earliest described occurrence of a toxic bloom of blue-green algae was reported by Francis (5) in 1878 in Australia. Since then toxic water blooms have been reported from Argentina, Canada, Israel, Russia, South Africa, and the United States. Various investigations and reports have been reviewed by several authors (4, 7, 9, 22, 23, 27).

In Canada, the earliest published report is by Howard and Berry (15) who reported that Anabaena spp. had been responsible for the poisoning of cattle at Fraser Lake in Ontario in 1924. Stewart, Barnum, and Henderson (29) reported cattle deaths which occurred in 1948 and 1949. The cattle had drunk water from Sturgeon Lake, Ontario, which had a heavy concentration of blue-green algae including Microcystis aeruginosa and Anabaena spp. Oral feeding and intraperitoneal injections of these algae or their filtrates killed bioassay animals.

O'Donoghue and Wilson (21) reported suspected algal poisoning from the vicinities of Duck and Baptiste lakes about one hundred miles north of Edmonton, Alberta. Cattle, horses, pigs, turkeys, geese and chickens were reported to have died or been sick for a period of time. MacDonald (18) recorded the deaths of 14

cattle pastured near a large Alberta lake. Intraperitoneal injections of algae into mice killed some a few minutes to 1½ hours later.

McLeod and Bondar (20) reported cases of suspected algal poisoning in Manitoba. In 1945, a horse, several calves, two pigs, and a cat died within an hour after consuming algae concentrated in the water of Lake Dauphin. In 1951, a horse and nine dogs died within an hour after drinking water from the lake. Bossenmaier et al. (2) reviewed reports and experimental work on duck sickness at Whitewater Lake, Manitoba. Heavy mortality of wild ducks occurred in 1949, 1950, and 1951 during the summer and fall. Experimental work indicated the presence of Clostridium botulinum and heavy scums of Aphanizomenon flos-aquae and Polycystis (Microcystis) aeruginosa. Intraperitoneal injections of algae into mice killed them as rapidly as 45 minutes after injections. They concluded that both botulinus organisms and blue-green algae may be involved in duck sickness.

The earliest known report of algal poisoning in Saskatchewan came from Hackl (12). In August 1954, a number of pigs died after drinking scum-covered water in Hoodoo Lake near St. Benedict. Piglets nursing on their dead mother also died. Cattle which drank from the lake were unaffected. A sample collected from the surface scum consisted entirely of Anabaena flos-aquae. Major outbreaks of algal poisoning occurred in 1959. Senior (28) reported the deaths of 30 dogs and one goose "at a Qu'Appelle lakeside resort" in late June. The algae present in the water

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were Aphanizomenon flos-aquae, Microcystis flos-aquae (aeruginosa) and Anabaena flos-aquae. Three cows died near Weyburn after drinking water containing Aphanizomenon and Anabaena. Dillenberg and Dehnel (3) reported on the same cases. Intraperitoneal injections of algal scum killed mice in 60 to 90 minutes. Three cows and six dogs were also reported dead at Buffalo Found Lake with blue-green algae presumed the causative agent. Dillenberg and Dehnel (3) also attributed a number of incidents of human sickness to blue-green algae. The algae were apparently ingested by people while they were swimming.

Six species of blue-green algae have been reported to be toxic (4). These are: Anabaena flos-aquae (Lyngb.) de Bréb., Aphanizomenon flos-aquae (L.) Ralfs, Microcystis aeruginosa Kuetz. emend. Elenkin, Nodularia spumigena Mertens, Gloeotrichia echinulata (J. E. Smith) Richter, and Coelosphaerium kuetzingianum Naegeli, using Prescott's (25) classification. These species are widely distributed in Saskatchewan lakes but only the first three species grow abundantly enough to produce blooms and scums (14).

The nature of the toxins produced by blue-green algae has become known within the last decade. Hughes, Gorham and Zehnder (16) found two toxic factors present in a unialgal culture of Microcystis aeruginosa. One of these, called the "slow death factor", caused death of mice in 4-48 hours and was later attributed to bacteria associated with the algae (7). The other factor caused death within one to two hours after intraperitoneal injection into mice and was therefore referred to as the "fast death factor". This toxin was present in both algal cells and filtrates (7). The pure "fast death factor" was ioslated by Bishop, Anet and Gorham (1). This cyclic polypeptide has been called microcystin by Konst et al. (17) who outlined the effects on a series of test animals.

Anabaena flos-aquae was first cultured from bloom samples obtained by the author from Burton Lake, Saskatchewan (10). This alga has also been associated with fast and slow deaths but the fast deaths occur much more rapidly than

those caused by *M. aeruginosa*. Gorham (8) referred to this toxin as a "very fast death factor". Although it has not been specifically identified, it is an amine of low molecular weight (26).

A study of blue-green algal toxicity in the environment was part of a larger ecological study of bloom-forming blue-green algae begun by the author in 1959. The impetus for the study of toxicity was given by the major outbreaks of algal poisoning in Saskatchewan during that year. The study was designed to determine the species involved in toxicity, the distribution of toxicity and a simple field method for the detection of toxic algae.

#### METHODS AND MATERIALS

Blooms and scums of algae were collected from lakes extending from Waskesieu Lake in Prince Albert National Park in the north to lakes of the Qu'Appelle River system in the south, totalling more than 50 lakes in all. These lakes varied tremendously in regard to their chemical nature (13, 14). Scum was collected from the lake surface and a plankton net (68 meshes/cm) was used to collect blooms from the open lake by drawing it through the water.

A one ml Tuberculin syringe equipped with a No. 26 needle was used to inject 20-35 gram male white mice intraperitoneally with concentrated algae or their filtrates. Doses up to one ml per mouse were used. In the field, injections were made immediately after collection without any special treatment of the algae. If samples were incubated, refrigerated or frozen, after being brought into the laboratory, they were brought to room temperature prior to injection. The mice were observed closely for an hour after injections and periodically thereafter. Algae-free waters from the respective lakes were used as controls.

## RESULTS

A summary of toxicity tests carried out by the author, using predominantly Anabaena flos-aquae, are shown in Table I. Only those tests where some physical effects on the mice were noted are recorded. Rapid deaths (within 2–50 minutes) occurred with algae from three

TABLE I Summary of Toxicity Tests with Anabaena flos-aquae Predominant in Algal Scums

Lake         Collection         Fraction tested         I.P. dose         Response         Symptoms           Katepwa         19/7/60         Plankton tow         0.5         2/2 survived         No obvious symptoms includated 1 days, includated 1 days, includated 1 days         0.5         1/1 died; 6 hr.         No obvious symptoms symptoms includated 1 days           Burton         14/9/60         Scum filtrate         0.5         1/2 died; 26 hr.         Severe abdominal contract sight partiylists of hind recovery 2 hr.           Culture at 25 °C.         1.0         2/2 survived         Inactive; day isk; recovered next day isk; recovered next day.           Scum filtrate         0.5         2/2 died; 7, 10 min.         Convulsions           Scum filtrate         0.5         2/2 survived         Inactive; day isk; recovered next day.           Washed algal scum         0.5         2/2 survived         Inactive; paralyzed hind I has; inactive; paralyzed hind I has; inactive; paralyzed hind I Refrigerated scum         0.5         2/2 survived         Inactive; paralyzed hind I has; inactive; paralyzed hind I has; inactive; paralyzed hind I Refore death thousen some 1.0         2/2 died; 12, 15 min.         Convulsions prior to death to death contractions just to death contractions just to death solution						The state of the s
19/7/60   Plankton tow   0.5   2/2 survived	Lake	Collection date	Fraction tested	I.P. dose ml/mouse	Response	Symptoms
Refrigerated 10 days,   0.5   1/1 died; 6.5 hr. at 31 °C.	Katepwa	19/2/60	Plankton tow	0.5	2/2 survived	
Frozen, thawed  9.5 1/1 died; 6 hr. scum  14/9/60 Scum filtrate  0.2 1/2 died; 26 hr. Incubated scum  (72 hr. at 25 °C.)  Culture at 25 °C.  1.0 2/2 survived  25/9/60 Fresh scum  0.5 2/2 died; 7, 10 min. Scum filtrate  0.5 2/2 survived  Washed algal scum  0.5 2/2 survived  Refrigerated scum  0.5 2/2 survived  Refrigerated scum  1.0 2/2 died; 12, 15 min.  Frozen, thawed scum  1.0 2/2 died; 6 min.  Decomposed scum  1.0 2/2 survived  2/2 survived  2/2 survived  2/2 survived			Refrigerated 10 days, incubated 1 day at 31 °C.	0.5	1/1 died; 6.5 hr.	No obvious symptoms
14/9/60       Scum filtrate       0.2       1/2 died; 26 hr.         Incubated scum       0.5       1/2 died; 13 hr.         (72 hr. at 25 °C.)       1.0       2/2 survived         25/9/60       Fresh scum       0.5       2/2 died; 7, 10 min.         Scum filtrate       0.5       2/2 survived         Washed algal scum       0.5       2/2 survived         Refrigerated scum       0.5       2/2 survived         Refrigerated scum       1.0       2/2 died; 12, 15 min.         Frozen, thawed scum       1.0       2/2 survived         solution       1.0       2/2 survived			Frozen, thawed scum	0.5	1/1 died; 6 hr.	
Incubated scum       0.5       1/2 died; 13 hr.         (72 hr. at 25 °C.)       1.0       2/2 survived         Culture at 25 °C.       1.0       2/2 survived         Fresh scum       0.5       2/2 survived         Washed algal scum       0.5       2/2 survived         Refrigerated scum       0.5       2/2 survived         Refrigerated scum       1.0       2/2 died; 12, 15 min.         Frozen, thawed scum       1.0       2/2 died; 6 min.         Decomposed scum       1.0       2/2 survived         solution       1.0       2/2 survived	Burton	14/9/60	Scum filtrate	0.2	1/2 died; 26 hr.	
Culture at 25 °C.1.02/2 survivedFresh scum Scum filtrate0.52/2 died; 7, 10 min.Scum filtrate0.52/2 survivedWashed algal scum0.52/2 survivedRefrigerated scum0.52/2 survivedRefrigerated scum1.02/2 died; 12, 15 min.Frozen, thawed scum1.02/2 died; 6 min.Decomposed scum solution1.02/2 survived			Incubated scum (72 hr. at 25 °C.)	0.5	1/2 died; 13 hr.	
Fresh scum         0.5         2/2 died; 7, 10 min.           Scum filtrate         0.5         2/2 survived           Washed algal scum         0.5         2/2 survived           Refrigerated scum         0.5         2/2 survived           Refrigerated scum         1.0         2/2 died; 12, 15 min.           Frozen, thawed scum         1.0         2/2 died; 6 min.           Decomposed scum         1.0         2/2 survived           solution         1.0         2/2 survived			Culture at 25 °C.	1.0	2/2 survived	Severe abdominal contractions, slight paralysis of hind limbs, recovery 2 hr.
Scum filtrate 0.5 2/2 survived  Washed algal scum 0.5 2/2 survived  Refrigerated scum 0.5 2/2 survived  Refrigerated scum 1.0 2/2 died; 12, 15 min.  Frozen, thawed scum 1.0 2/2 died; 6 min.  Decomposed scum 1.0 2/2 survived solution 1.0 2/2 survived		25/9/60	Fresh scum	0.5	2/2 died; 7, 10 min.	Convulsions
Washed algal scum0.52/2 survivedRefrigerated scum0.52/2 survivedRefrigerated scum1.02/2 died; 12, 15 min.Frozen, thawed scum1.02/2 died; 6 min.Decomposed scum1.02/2 survivedsolution2/2 survived			Scum filtrate	0.5	2/2 survived	Inactive, sick; recovered next day
Refrigerated scum0.52/2 survivedRefrigerated scum1.02/2 died; 12, 15 min.Frozen, thawed scum1.02/2 died; 6 min.Decomposed scum1.02/2 survivedsolution2/2 survived			Washed algal scum	0.5	2/2 survived	Occasional abdominal contractions; paralyzed hind limbs; inactive; gradual recovery
Refrigerated scum 1.0 2/2 died; 12, 15 min.  Frozen, thawed scum 1.0 2/2 died; 6 min.  Decomposed scum 1.0 2/2 survived solution			Refrigerated scum	0.5	2/2 survived	Inactive; paralyzed hind limbs
Frozen, thawed scum 1.0 2/2 died; 6 min.  Decomposed scum 1.0 2/2 survived solution			Refrigerated scum	1.0	2/2 died; 12, 15 min.	Violent contractions just before death
Decomposed scum 1.0 2/2 survived solution			Frozen, thawed scum	1.0	2/2 died; 6 min.	Convulsions prior to deaths
		10/10/60	Decomposed scum solution	1.0	2/2 survived	No apparent effect

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TABLE I (concluded)

	Collection		I P dose		
Lake	Date	Fraction Tested	ml/mouse	Response	Symptoms
Buffalo Pound	30/5/61	Refrigerated scum	1.0	2/2 died; 13 min.	Lethargic; convulsions prior to death
	19/9/61	Fresh scum	1.0	2/2 died; 50 min. 15 hr.	
	2/7/61	With Microcystis	1.0	2/2 survived	None
Hoodoo	5/6/61	Refrigerated scum	1.0	2/2 died; 31, 33 min.	Lethargy; hind limb paralysis
Burton	13/6/61	Scum precipitate	1.0	2/2 died; 7, 19 hr.	Lethargic
	20/6/61	Fresh scum	1.0	2/2 died; $2$ min.	Stiff-legged jumps prior to death; convulsions
	18/8/61	Fresh scum	1.0	2/2 died; 10 min.	Violent leaps before death
Last Mountain	19/9/61	Plankton tow	1.0	2/2 died; 4, 18 hr.	None apparent
Buffalo Pound	13/6/62	Refrigerated scum	0.5	1/1 died; 10 min.	Lethargy; convulsions just before death
		Refrigerated scum	0.25	1/1 died; 11 min.	do.
			0.1	1/1 died; 19 min.	do.
		Plankton tow	0.5	2/2 died; $5$ , $9$ min.	do.
		Plankton tow	0.25	1/1 survived	Lethargy; gradual recovery
Buffalo Pound	2/9/2	Algal scum	1.0	2/2 died; 2, 2.5 min.	Violent jumps, just prior to death
Buffalo Pound	14/6/66	Fresh scum	1.0	1/1 died; 3.5 min.	None apparent
			0.5	1/1 died; 9 min.	

lakes, Buffalo Pound, Burton and Hoodoo, and were presumably due to the "very fast death" factor. The typical symptoms of paralysis of the hind limbs and convulsions prior to death were usually present. On occasion, milder forms of these symptoms occurred without deaths ensuing. Similar tests were carried out on other occasions and on other lakes where the same alga was dominant but deaths failed to occur. Plankton tows in open water concentrated *Anabaena* sufficiently to cause fast deaths on only one occasion.

The summary of toxicity tests involving *Microcystis aeruginosa* as the dominant alga is shown as Table II. Only tests of scums and blooms which had some debilitating effect on mice are reported. Many more tests were made on scums of the lakes, and of others as well, with negative effects. The presence of "fast death" toxin occurred apparently on only one occasion: Hoodoo Lake on July 27, 1960. Slow deaths were of more frequent occurrence.

Although many toxicity tests were carried out using *Aphanizomenon flos-aquae*, no fast deaths occurred. Occasionally, slow deaths (5–48 hours) of mice took place. As this species forms more blooms than any other blue-green alga in Saskatchewan, many blooms and scums were tested.

Table III summarizes cases of the poisoning of animals where blue-green algae were the suspected causative agents. Occasionally positive toxicity tests noted in Table I coincided with livestock deaths listed in Table III, e.g., Buffalo Pound—late May, 1961; —June, 1966; Burton—June, 1961. Dr. Senior (personal communication) wrote that in the Grenfell and Kindersley cases "hemorrhagic enteritis" was symptomatic of algal poisoning (Table III), as he reported with respect to dogs in 1960 (28).

## Discussion

A variety of animals have died from suspected algal poisoning in Saskatchewan. The alga most frequently associated with these deaths is Anabaena flos-aquae while Microcystis aeruginosa appears to be less frequently involved. To date, toxic Aphanizomenon flos-aquae strains have not been grown in culture (9) and are

probably not involved in animal poisoning in Saskatchewan.

The incidence of livestock deaths is almost invariably associated with the collection of algal scums along the shore by light wind action. This is always preceded by calm weather when the blue-green algae rise to the surface of the water. As the algae are concentrated near the shore smaller animals are more likely to imbibe water containing masses of algae than larger animals which wade out further. This is apparently the reason why older cattle are not poisoned while calves and dogs are, even though drinking from the same body of water. Since blue-green algae are not concentrated sufficiently in the open water as compared to scums along the shore (as shown by relatively non-toxic plankton tows), owners of livestock must keep them away from the concentrated scums. Careful periodic observation of the water condition and the wind direction and velocity are important in preventing deaths.

Another important aspect related to algal poisoning is the occurrence of strains of a blue-green algal species which have different properties (10, 19). Some strains are toxic while others are not. Strains may be mixed in natural situations or separate. The differences are not obvious visually or microscopically so that every bloom must be treated as if it were toxic. Toxicity may be very rapidly confirmed by the method of injecting mice as outlined previously.

An interesting case of apparent algal poisoning was the fish kill in Burton Lake in July, 1961 (Table III). Oxygen concentrations were adequate while the fish were dying. Anabaena flos-aquae scums were precipitated on the bottom where perch normally feed. The fish stomachs were filled with fish fly larvae (Chironomus sp.) which were very green in color. It seems probable that the fish obtained the toxic "fast death factor" indirectly by eating the insect larvae. Prescott (24) has, however, reported fish killed by hydroxylamine, a product of algae decomposition.

The problem of "duck sickness" has long been disputed with regard to its precise cause. Gorham (7) found the *Microcystis* toxin was not toxic to ducks. He

TABLE II Summary of Toxicity Tests with  $\it Microcystis$  aeruginosa Predominant in Algal Scums

Lake	Collection date	Fraction tested	I.P. dose ml/mouse	Response	Symptoms
Hoodoo	18/2/60	Algal scum Incubated scum (31° C., 24 hr.)	0.2	1/1 survived 1/1 died: 2.5 hr.	
Hoodoo	27/7/60	Frozen, thawed scum	00 101	1/1 died; 7.5 hr.	No obvious symptoms
		Incubated scum Frozen thawed scum		1/1 died; 6 hr.	Periodic convulsions, eyes closed, shaking
	_	Refrigerated scum Algal scum	0.00 0.00	2/2 survived 2/2 died: 5 17 hr	Partial paralysis of hind limbs initially
Разсии	8/9/60	Algal scum		1/2 died; 48 hr.	
Hoodoo		Algal scum		2/2 died; 2, 7 nr. 2/2 died, 12, 19 hr.	

TABLE III

CASES OF SUSPECTED ALGAL POISONING OF ANIMALS IN SASKATCHEWAN IN 1961 TO 1967.

Location	Date	Animals Affected and Effects	Suspected Causative Agents
Buffalo Pound Lake (Moose Jaw)	May 28, 1961	20 dogs died after drinking water from lake	Anabaena flos-aquae—scums present
Burton Lake (Humboldt)	June 11, 1961	2 cattle died, 2 others sick (Gaskin (6))	Heavy blooms and scums of Anabaena flos-aquae present
	June 25, 1961	1 cow died (Gaskin (6))	Same. Tested toxic on June 20.
	July 3-9, 1961	Heavy perch kill	Anabaena bloom and precipitate
Proctor Lake (Hanley)	Early September 1961	Heavy wild duck mortality; paralysis prior to death	Blue-green algae or botulism; both present
Buffalo Pound Lake	May 26, 1962	Three dogs died	Algae (report in Regina Leader Post)
*Crooked Lake (Grenfell)	July 10, 1964	1 dog died (confirmed); 4 dogs died (hearsay)	Anabaena flos-aquae abundant
*Dugout near Kindersley	July 13, 1964	20 calves sick; 1 died	Anabaena, Aphanizomenon and Nodularia present
Lake near Paradise Hill	August 10, 1965	17 head of cattle died after drinking from lake	Anabaena flos-aquae akinetes, Aphanizomenon and Microcystis aeruginosa in water sample
Buffalo Pound Lake	June 13, 1966	2 calves and $1$ dog died	Anabaena flos-aquae predominant in bloom
*Small reservoir near Indian Head	June 7, 1967	25 pigs died with vomiting and convulsions before death	Anabaena species bloom

\*Information supplied by Dr. V. E. Senior, Provincial Veterinarian, Regina.

(9) reported that Olson dosed ducks with toxic Anabaena bloom which killed ducks rapidly in one to two hours with symptoms similar to those attributed to duck sickness. One of these symptoms is limberneck which is also characteristic of botulinus poisoning. Botulinus antisera failed to counteract the poison. This work suggests that duck sickness may be caused by blue-green algae as well as botulism, a conjecture made earlier by Bossenmaier et al. (2). A toxicity test of algal scum (if present) would aid in testing this hypothesis.

The effect of toxic and decomposing algae on human users and consumers is a perennial problem. Since the known toxins are endotoxins they are not released in any large amounts unless cell lysis occurs. This happens when scums accumulate and decomposition ensues due to high light intensity and bacterial action. This tends to occur near shore and swimming should be discouraged when scums accumulate. However, ingestion of algae and their toxins tends to be accidental rather than purposeful. For domestic use, water supplies are generally drawn from well off shore and below the surface. There is thus little possibility of algal toxins occurring in the water supply in sufficient quantities to be harmful to human health. However, even if this should happen, the toxins are inactivated by standard water treatment procedures (11).

Various antidotes for algal poisoning have been suggested by different authors (3, 6, 18, 21, 22) but the successes reported were probably fortuitous. Animals receiving sublethal doses, as indicated earlier, recover without treatment. No antidote has yet been discovered for the two blue-green algal toxins.

Direct control of algae with algicides is only feasible when the water bodies are small, i.e., dugouts and small lakes. Marginal treatment of large lakes is of temporary utility only since the effect is destroyed with wind action. After treatment the toxins are released and livestock must not be allowed to drink there for at least 24 hours.

In general, algal intoxication of man and domestic animals may be prevented by utilizing water sources other than those covered with algal scums. However, there is at present no way by which poisoning of natural populations of fish, waterfowl, and wildlife by toxic blue-green algae may be prevented.

## SUMMARY

The effects of toxic blue-green algae on mammals and waterfowl in Canada were reviewed, and documented for Saskatchewan. Toxicity tests on many algal scums were carried out. Two species, Anabaena flos-aquae and Microcystis aeruginosa, appear to be the only ones significant in algal intoxication in Saskatchewan lakes.

The origin and nature of blue-green algal toxins were described. Possible implications for humans and the prevention of algal intoxication were discussed. If proper preventive measures are taken, there is little likelihood that man or his domestic livestock will suffer. On the other hand, there is no known method by which deaths of algal origin may be prevented in natural populations of fish, waterfowl or wildlife.

## RÉSUMÉ

L'auteur a passé en revue les publications sur les effets toxiques des algues bleu-vert sur les mammifères et les oiseaux aquatiques du Canada, et il s'est renseigné plus particulièrement sur les troubles qui se rencontrent en Saskatchewan. Il a analysé les résidus de plusieurs sortes d'algues pour en déterminer le degré de toxicité. Il semble que, seules, deux espèces, Anabaena flos-aquae et Microcystis aeruginosa, qui se rencontrent dans les lacs de la Saskatchewan, renferment des éléments toxiques.

Il décrit l'origine et la nature des toxines des algues bleu-vert. Il envisage la possibilité d'intoxication chez l'homme et il suggère des moyens de prévention. Pourvu qu'on ait recours à des mesures prophylactiques adéquates, il est peu probable que l'homme et les animaux domestiques en soient incommodés. D'autre part, dans le milieu naturel, on ne connait pas de mesures préventives contre l'intoxication par les algues chez les poissons, chez les oiseaux aquatiques ou chez les animaux sauvages.

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